The Structure and Formation of the Crystalline Style of *Telescopium telescopium* (Linnaeus)

(Gastropoda : Prosobranchia)

BY

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(4 Text figures)

INTRODUCTION

THE WHELK *Telescopium telescopium* (Linnaeus, 1758) is an Indo-Pacific species which inhabits mangrove swamps. It is usually found in, or in very close proximity to pools of standing water and is able to tolerate a wide range of salinities ranging from 15 parts per thousand up to full strength sea water in the environment. These animals possess a crystalline style which is present even in the smallest specimens. There is no variation in style size during the tidal cycle unlike many style bearing lamellibranchs where the style may break down and reform (GRAHAM 1949), and it persists even when the animal is starved for considerable periods.

The style of *T. telescopium* was first described by SESHAIYA (1932) and Swaminathan analysed it for amino acid content (SWAMINATHAN 1958 *in* FLORKIN 1966).

Within the crystalline style bearing molluscs there is considerable variation in style sac morphology. There are recognised to be three major divisions which were first proposed for lamellibranchs (MATTHAIS 1914 *in* MACKINTOSH 1925):—

(a) Those in which the style is completely free within the intestine or in wide communication with it.

(b) Those in which the typhlosoles have almost completely grown together so that the intestine and the style sac communicate only by a narrow slit.

(c) Those in which the style sac is completely separate from the intestine. *Telescopium telescopium* falls into this category.

Recently, DRISCOLL (1972) discussed the alimentary tracts and styles of two mesogastropods from North America, *Batillaria zonalis* Bruguière, 1792, and *Cerithidea californica* Haldeman, 1840. The aim of the present paper is to describe the structure of the style sac and style of *Telescopium telescopium* and to suggest where and how the style is formed.

MATERIALS AND METHODS

For wax sections, specimens of style sac and style within were fixed in Bakers formal-calcium. One constant difficulty was to prevent the style from shattering during sectioning. This was overcome by soaking the surface of the block prior to sectioning with Mollifex or Baker's fluid for about thirty minutes and this allowed up to a dozen complete sections to be taken from the block. After dewaxing in xylene and cleaning in absolute ethanol, sections were treated with 0.5% solution of celloidin in 50:50 ether ethanol which was then air dried and allowed to harden in 95% ethanol. This effectively prevented the sections and in particular the style from detaching from the slide during subsequent processing and staining. From here on the sections could be stained without section loss.

Routinely, sections were stained with haematoxylin and eosin, alcian blue, periodic acid Schiff, and Lison's alcian blue chloratine fast red. For histochemical analysis of style sac mucins, sets of sections were treated according to the following schedule.

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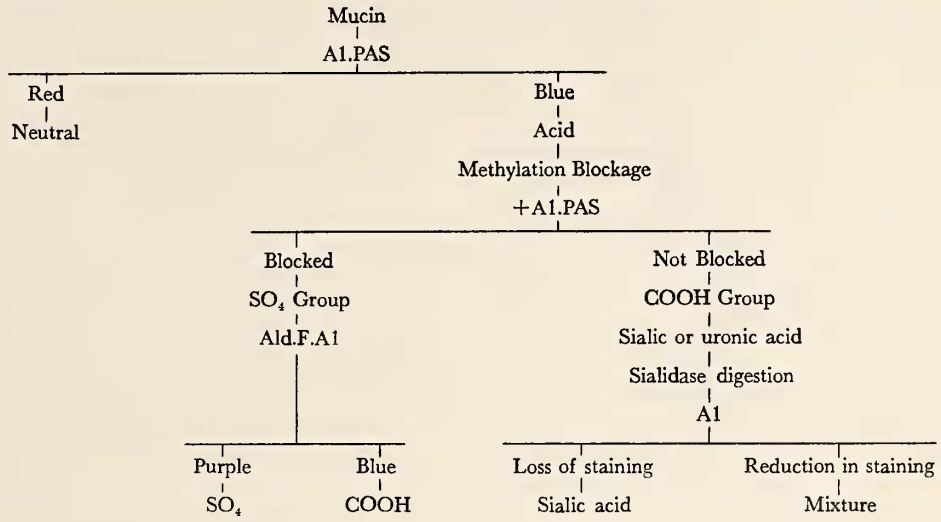

PAS = Periodic Acid-Schiff
Al = Alcian Blue
Ald.F = Aldehyde fuchsin

For epoxy resin embedding, small pieces of tissue from the distal tip of the style sac and from the style sac epihelium were initially fixed in a mixture of paraformaldehyde-gluteraldehyde-acrolein in Sorenson's phosphate buffer pH 7.2 for 30 minutes. They were then washed for three hours in buffer, post fixed in 1% osmium tetroxide in the same buffer for one hour and washed for three hours. The material was dehydrated in graded acetone solution prior to infiltration with and subsequent embedding in Epon. Sections were cut from the resulting blocks with a glass knife on an LKB Huxley ultramicrotome.

Test animals were injected in the foot with a 0.5% solution of iron saccharate in sea water following the method of YONGE (1926). One half cc of this solution was injected over a period of fifteen minutes to reduce physiological shock. After intervals of two, four and six hours, the style sacs were removed and fixed in Baker's formol calcium and sections 10 μm in thickness prepared. These were stained to detect Perl's Prussian Blue reaction (DRURY & WALLINGTON, 1967) and counter stained with neutral red.

RESULTS

Digestive Anatomy

The digestive tract of *Telescopium telescopium* is shown diagrammatically in Figure 1. In life, both it and the style sac are coiled. The oesophagus is a long straight thin walled tube. The stomach is globular to pear shaped from which arises a spiral caecum attached to which is the digestive gland. After leaving the stomach, the digestive tract loops back on itself once and from then on forms the rectum and anus which discharges into the mantle cavity. The style sac connects with the antero-lateral border of the stomach. The stomach, intestinal loops and the style sac are embedded in a large mass of connective tissue.

Structure of the Style

The style is cylindrical in shape, its length and diameter varying with the length of the animal so that an animal measuring 8 cm in length taken along the ver-

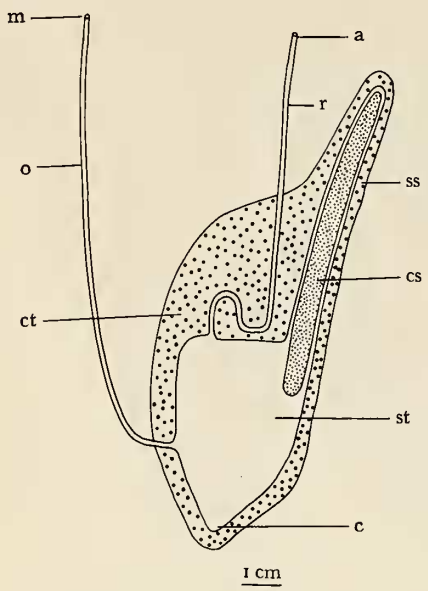

Figure 1

The digestive tract of *Telescopium telescopium*

a – anus c – caecum cs – crystalline style
ct – connective tissue m – mouth o – oesophagus
r – rectum ss – style sac st – stomach

tical axis through the columella would have a style approximately 8 cm long by 3 mm in diameter. The style is quite flexible, transparent and sharply tapered at the distal end and bears a protuberance at the extreme distal tip. The core of the style is composed of amorphous material which constitutes about ⅕ of the total diameter of the style. The core is surrounded by many concentric layers of much harder material. These appear parallel with one another and are concentrically, not spirally arranged, they are not, however, parallel with the longitudinal surface of the style so that the style is made up of numerous cones stacked one on top of each other. One aberrant specimen of the many dozens examined had a style in which there were two amorphous cores.

Structure of the Style Sac

The proximal end of the style sac is connected to the stomach by a narrow slit which is lined by a ciliated columnar epithelium. In the middle region of the style sac this slit is no longer present but a ventral groove is present which persists for the remainder of the style sac. One lip of the ventral groove is enlarged and probably represents the remnants of one of the two typhlosoles present in such forms as *Batillaria zonalis* (Driscoll, 1972). The ventral groove, as seen in *Telescopium telescopium* is, according to the classification of Kubomura (1957), homologous with the slit between the two typhlosoles. The style sac proper is completely lined by a ciliated cuboidal epithelium of uniform size except in the typhlosole and ventral groove regions where the cells are elongated but the cilia are shorter and sparser (Figure 2).

Structure of the Style Formation Region

The diagram (Figure 3) shows the positions and thickness of the tissues present in this region. At level (A) is encountered the typical connective tissue which surrounds much of the alimentary canal and the whole of the style sac (Figure 1). The histological detail of this tissue can be seen in Figure 4. At level (B) an area of

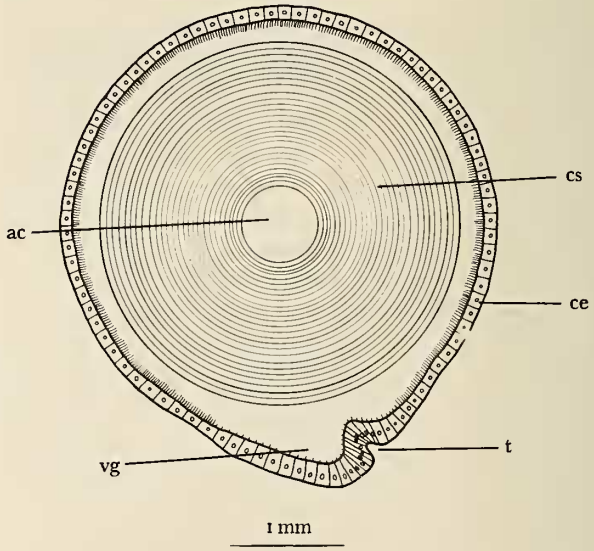

Figure 2

Transverse section of the style sac and style

ac – amorphous core of the style
ce – cuboidal epithelium of the style sac t – typhlosole
cs – crystalline style concentric layers vg – ventral groove

strongly alcianophilic material forms. The position of this material in section is indicated in the drawing. This stained area appears to have no obvious cellular structure when examined with an optical microscope. At level (C) a region of equally featureless PAS positive material appears as a small patch within the alcianophilic material. At level (D) the lumen of the style sac is first visible and

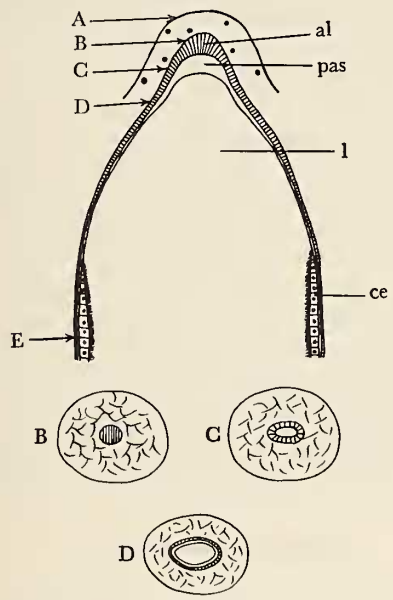

Figure 3

Diagrammatic reconstruction of the style sac distal tip
The lower sketches indicate the appearance of stained material in the sections

A – E (arrowed) levels of sections referred to in the text
al – alcianophilic material (hatched) l – lumen of style sac
ce – cuboidal epithelium of the style sac
pas – periodic acid Schiff positively reacting material

this rapidly expands over a distance of about 2 mm to the maximum size of the style sac which persists as such for the whole of its length. The style sac epithelium first becomes apparent in the position indicated in Figure 3 and at this point the typhlosole and ventral groove also form. The alcianophilic and PAS positive material persist as thin subepidermal layers for the whole length of the style sac.

Figure 4 shows the histological detail of the style sac epithelium revealed by examination of very thin (1 μm) optical sections stained with toluidine blue. The epithelial cells are cuboidal in shape, have a microvillous border and many cilia. At intervals are groups of cells showing metachromatic inclusions some of which can be seen to be discharging their content in the manner of holocrine glands. At intervals there are minute canals which penetrate through the epithelial layer from the subepithelial region to the lumen of the style sac. The subepithelial layer contains a large number of deeply stained elongated bodies. It is not possible to distinguish any cellular structure in these optical sections. These are the regions which show strong alcianophilia and positive response to the PAS test. Underlying this is the so called connective tissue. This region is interesting on account of the large number of non cellular cavities. Some of these cavities show rings of concentric material of much harder consistency. These concretions are more numerous in the region of the slit between style sac and stomach than elsewhere and possibly support the large mass of connective tissue in this region. If the glass knife used to cut sections of this tissue is not new with a very sharp cutting edge, these areas are torn out of the sections. The other cavities contain a soft secretion which stains lightly with toluidine blue. These are not lipid deposits because there is no reaction to osmic acid in which the tissues were originally fixed.

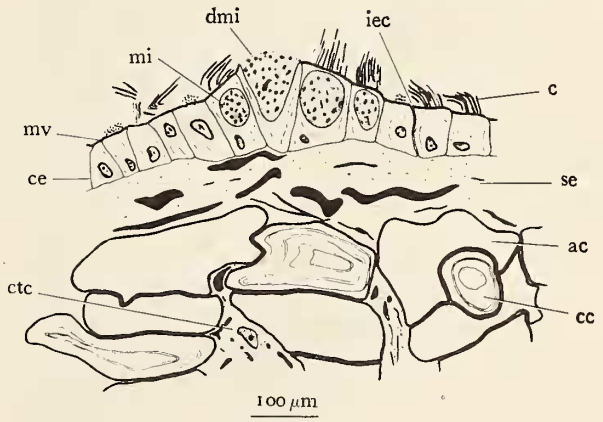

Figure 4

Drawing of the style sac epithelium revealed by 1 μm sections stained with 1% toluidine blue and examined under oil immersion phase contrast illumination

ac – amorphous cavity cc – cavity with concentrically layered concretions c – cilia (note some are shown in transverse section) ctc – connective tissue cellular material
iec – inter-epithelial cell canal dmi – discharging metachromatically staining cell inclusion mi – metachromatically staining cell inclusion mv – microvilli se – subepithelial layer

DISCUSSION

Most authors have stated that the crystalline style is secreted by the cells of either the ventral groove or the typhlosole (Mackintosh, 1925; Yonge, 1926, 1932; Dris-

COLL, 1972). A style secreted along the length of the style sac should have a tapered and spiral structure and styles such as this have been described by Driscoll in *Cerithidea californica* and *Batillaria zonalis*. The style of *Telescopium telescopium* cannot be secreted in this manner because of the uniform diameter along the whole of its length with the exception of the extreme distal tip. If the style were secreted along the whole of its length this would necessitate continuous secretion and resorption which seems very unlikely. The style in cross-section consists of a large number of very thin concentric layers with no indication of a spiral arrangement. The centre of the style consists of amorphous, much softer material. The whole structure is concomitant with a series of cones fitting on top of each other.

It is considered that the distal end of the style sac is the sole region of style secretion. The amorphous core of the style must be secreted at the apex of the dome at the extreme end of the style sac. The style itself must be secreted by the alcianophilic and PAS positive material. Secretion is probably rhythmical so that one layer or cone of material is added to the end of the style at a time with the style rotating continuously or sporadically at the time of each secretion. It is presumed that the hyaline style material is in a fluid form which hardens when it comes into contact with the existing style or with the fluid in the style sac.

The series of histochemical tests carried out showed that a wide variety of muco-substances are present; acid, neutral, carboxylated and sulphated. This does no more than indicate the chemical complexity of the style which must be structurally composed of proteins (BRIGHT 1958) although this aspect was not examined in the present work.

It is difficult to observe the actual rotation of the style in *Telescopium telescopium* because of the morphology of the animal and the convoluted course of the style in the living animal. By inference it can be demonstrated that the style does rotate. The style sac typically is heavily ciliated as are the style sacs of animals where style rotation can be more readily observed. (DRISCOLL, 1972). The animal possessing a style with two centres of secretion also provided some evidence. In this animal, at the point where the two centres of secretion meet the typhlosole lies in such a position that a line drawn from the typhlosole along a radius of the style bisects a line drawn between the two style centres. Further down the style, the typhlosole and style cores lie almost along the style radius showing that the style must have rotated. The style cores must therefore trace a double helical pathway.

It is reasonable to assume that the typhlosole and ventral groove are secretory centres. Examination of sections of material fixed four hours after the injection of 0.5% iron saccharate showed that there was a significant reaction to Perl's Prussian blue reaction in these regions. These are not, however, regions of style secretion but are probably important centres for the production of digestive enzymes which become adsorbed on to the style. Although the investigation of digestive enzymes did not form a part of the present work, tests were carried out to see if amylase enzymes were present, as seems to be the case in most crystalline styles so far examined. Despite repeated attempts the presence of amylases could not be demonstrated. There are many such anomalous reactions from this animal which merit further investigation.

ACKNOWLEDGMENTS

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